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DEEP STOPS DURING DECOMPRESSION IN A SWINE MODEL OF DECOMPRESSION SICKNESS

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The experiments reported herein were conducted according to the principles set forth in the current edition of the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animals Resources, National Research Council.

This technical report has been reviewed by the NMRI scientific and public affairs staff and is approved for publication. It is releasable to the National Technical Information Service where it will be available to the general public, including foreign nations.

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13. ABSTRACT *(Maximum 200 words)*

In a porcine model of neurological and cutaneous decompression sickness (DCS), the effect of a deep, initial stop during decompression from a heliox dive was examined. In the first phase of the study, control (regular stops) pigs were dived in a dry chamber environment on a profile of 250 feet of seawater (fsw) for 30 min with 3 decompression stops: 10 min at 120 fsw; 20 min a 60 fsw; and 50 min at 20 fsw. The experimental (deep stops) group underwent a profile of the same depth and bottom time, but the decompression consisted of 6 stops: 1 min at 220 fsw; 2 min at 190 fsw; 7 min at 160 fsw; 10 min at 120 fsw; 20 min at 60 fsw; and 30 min at 20 fsw. Each group comprised 31 pigs. Animals were observed postdecompression for the onset of neurological and cutaneous DCS. In the regular stops group, 13 animals developed neurological DCS and 6 manifested cutaneous DCS. In the deep stops group, 13 pigs developed neurological DCS and 7 cutaneous DCS. Therefore, no statistically significant difference was detected in the incidence of either neurological or cutaneous DCS for the deep stop profile, even though it had 8 min less total decompression time (TDT). In the second phase of the experiment, the control profile from the first phase was changed so that its last decompression stop was 42 min at 20 fsw, giving both the regular stop and deep stop profiles the same TDT. The deep stop profile was unchanged. Each group in the second phase comprised 29 animals. In the regular stops group, 16 pigs developed neurological DCS

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and 11 developed cutaneous DCS. In the deep stop groups, 13 pigs developed neurological DCS and 13 cutaneous DCS. The difference in incidence for neurological DCS (0.6 vs 0.5) was not significant using chi-squared analysis with Yates' correction ($\chi^2 = 0.28$; $p > 0.10$). In both phases of the study, the incidence of cutaneous DCS was slightly higher in the deep stops group, but neither of these differences were statistically significant. The deep stops profile allows a reduction in TDT without an increase in incidence of DCS. However, it would also appear that the decompression could also be accelerated without the use of a deep stop.

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The experiments reported herein were conducted according to the principles set forth in the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council, National Academy Press, 1996.

INTRODUCTION

In the early 1900's, based on experimental work in goats, Haldane (1) proposed that the most efficient method of decompression was to use staged decompression such that no tissue in the body became supersaturated to the extent that bubbles would form. This led to the "2:1 ratio" concept: after a given pressure exposure, it was safe to reduce rapidly the pressure to about half of the original working pressure, then proceed in further stages with lengthening stops as the surface was approached. Haldane's method has been refined by successively more complex mathematical handling of theoretical gas tissue kinetics in order to explain the observed inaccuracies of the technique in practice, but most calculations share the underlying theoretical aim of preventing tissue supersaturation. A recent exception is the maximum likelihood method, which uses decompression incidence data, to predict the failure rate of tables based on Haldanian concepts.

Interestingly, there are several issues about which we know little. Although development of Haldane's (1) original concept has given us one way of devising largely safe decompression schedules, we do not know whether his concept is the best. Several authorities view schedules based on his theories as treatment tables in which the prolonged decompression stops near the surface are needed to eliminate the bubbles that formed in the preliminary rapid reduction in pressure. This has led to experiments that indicated that short, deep stops produce less need for long, shallow stops, yielding a net saving in total decompression time. Rigorous experimental demonstration of this phenomenon has not been reported, but this is now possible using our pig model.

METHODS

Subjects and Animal Husbandry

Juvenile, male, neutered, pure-bred, Yorkshire swine from a closed breeding colony (weight range 16-20 kg on delivery) were received as numbered littermates. On receipt, pigs were examined by a veterinarian and each fitted with an adjustable canine chest harness (Coastal Pet Products; Alliance, OH) to facilitate handling. All pigs were housed in pairs until catheterized, at which time they were separated and placed in different runs indoors. Water was freely available in each run and the minimum daily diet consisted of 2% by body weight of Purina Hog Finisher No. 50, which maintained a gradual weight increase with growth.

Pigs were in-house for about one week before diving. After an initial 24 h to adjust to their new surroundings, the animals were introduced into the laboratory environment. Each weekday they were transported from the animal care facility to the laboratory in plastic transport kennels (Vari-Kennel, R.C. Steele; Brockport, NY). All pigs were then habituated to the general laboratory conditions and to the 4 ft x 4 ft enclosure where they would be observed for signs of DCI after diving. Each pig was also familiarized with the compression chamber and with the noise of flowing gas experienced during the dive.

All pigs were trained to run on a modified laboratory treadmill (Marquette Electronics; Milwaukee, WI). Training was made easier if a novice pig first observed an experienced pig running on the treadmill. The best time for training was before the morning feeding, as gastro-colic reflex effects were reduced and feeding immediately after the treadmill session induced a Pavlovian response to training. After 3 - 4 sessions, most pigs ran easily on a 5% incline at a

speed of 4 mph for 5 min. More prolonged or strenuous training was avoided because physical conditioning had been found to reduce the risk of neurological decompression illness (DCI) (2).

Predive Preparation

Procedures were carried out with pigs passively restrained in a Panepinto sling (Charles River, Wilmington, MA), which cradled the animals' body while the legs hung down through holes. The slings were mounted on wheeled carts, permitting easy movement around the laboratory.

On the afternoon before their dive, pigs were anesthetized (IM ketamine, 400 mg; xylazine, 20 mg) and venous catheterization of an ear vein was performed. This enabled both venous blood sampling predive and rapid venous access if a pig developed DCS after diving. We used customized, 18-inch long, polyurethane catheters (Braintree Scientific Inc.; Braintree, MA) with 0.040" external / 0.025" internal diameter and an integral luer hub. After a cut-down onto the ear vein, the sterilized catheter was advanced 10 - 12 inches into the central thoracic veins, then lightly tied into the vessel. The cut-down incision was sutured closed and an injection port (Interlink System; Baxter, Deerfield, IL) was fitted to the catheter. Pigs were then given IV chloramphenicol, 500 mg, to reduce the risk of infection, and then the injection port was heparinized to maintain catheter patency overnight. The catheter was then firmly secured to the dorsum of the pig's ear using 2-inch woven, surgical, adhesive tape (National Patent Partnership; Dayville, CT). This type of tape proved the most reliable in confounding the pigs' attempts to remove it.

Before diving, each pig fasted overnight but had access to water *ad libitum.* In the morning, the pig was weighed, then placed in a Panepinto sling. The IV catheter was untaped

and predive blood samples were drawn. The catheter was then retaped to the ear. Handled gently, pigs tolerated these predive procedures with no or minimal complaint, making sedation or anesthesia unnecessary.

Dive Procedures

All pigs were dived once only, as described below. The compression chamber was a 66 ft x 30 ft cylinder (Bethlehem Corporation; Baltimore, MD). Each pig was dived while unrestrained in a transport kennel. The compression profile was controlled automatically by a computerized unit (Digital Programmer; Honeywell Corp., Phoenix, AZ) that responded to a pressure transducer in the chamber (Smart Transmitter, 900 Series, Honeywell Corp.) and driven automated valves that control compression and exhaust (SVF; Santa Anna, CA). The decompression profile was guided manually to follow a preprogrammed track displayed by a computerized unit. This displayed a real-time, plus or minus display calibrated as fsw off-track, and an accuracy of within ± 2 fsw off-track was consistently achieved during the decompression.

First Phase

Control Dives

A total of 31 pigs performed a dive on 80%/20% heliox (see Figure 1). The dive was to 250 feet of seawater (fsw) (765.8 kPa) for 30 min. Compression took 5 min 50 s: 2 min at 20 fsw/min (61 kPa/min) to 40 fsw; 1 min at 40 fsw/min (122 kPa/min) to 80 fsw; then 2 min 50 s at 60 fsw/min (183 kPa/min) to 250 fsw. Time at chamber bottom was 24 min 10 s, followed by decompression at 60 fsw/min with decompression stops of 10 min at 120 fsw, 20 min at 60 fsw, and 50 min at 20 fsw. Total decompression time (TDT) was 1 h, 26 min, 40 s.

Pigs were dived inside a transport kennel specially modified to be gastight, other than through inlet and outlet valves. Heliox (80%/20%) was flushed into the kennel at flow rates up to 100 l/min until a total volume of 500 l was flushed through the box. In order to insure this procedure produced an accurate flush, in a preliminary experiment, a set volume of 500 l heliox was flushed through the kennel measured on a flowmeter. Duplicate gas samples of 1.0 l each were then checked for $\%N_2$ by gas chromatography (Shimadzu GC14A Molsieve column), demonstrating < 6% N_2 in each sample. During each dive, after the initial flush, the kennel was then placed in the chamber, and the chamber pressurized on air while maintaining a positive flow of heliox into the kennel. This was intended to insure that the pigs breathed heliox throughout the dive, while achieving considerable economy in the use of heliox.

Deep Stop Dives

A total of 31 pigs underwent a dive on 80/20% heliox. The compression and bottom time for this profile was exactly the same as for the control profile. The decompression rate was again 60 fsw/min with 6 decompression stops: 1 min at 220 fsw; 2 min at 190 fsw; 7 min at 160 fsw; 10 min 120 fsw; 20 min at 60 fsw; and 30 min at 20 fsw. The procedure for flushing of the gastight transport kennel was exactly the same as that used for the control profile.

Each day, a matched litter pair was randomized by coin toss to either the control or the gas switch profile. The principal investigator was blinded to the profile dived by individual pigs. Diagnosis of neurological and cutaneous DCS was therefore made without the knowledge of the preceding dive profile.

Second Phase

Control Dives

A total of 29 pigs underwent the control dive. All aspects of the dives were the same as the control profile for the first phase except that the time of the last decompression stop was reduced by 8 min. This made the TDT of the control and experimental dives equal.

Deep Stops Dives

A total of 29 pigs underwent the deep stops profile. All aspects of the dive, including the pairing and randomization, were exactly the same as for the first phase of the study.

Postdive

On surfacing, all pigs were transferred from the chamber into the laboratory observation pen where they were closely observed. Behavioral features and constitutional signs such as lethargy were noted, but not considered alone to be evidence of DCS. Neurological signs such as limb weakness, paralysis, or ataxia were recorded and considered neurological DCS. Skin lesions were also recorded and considered cutaneous DCS. If any signs of distress were observed, pigs were sedated by diazepam, 5-10 mg IV, and observation was continued until 1 h postdive, whereupon a final assessment of skin DCI was made. At this point, affected pigs were first anesthetized by IV injection of ketamine (400 mg in 4 ml) and xylazine (20 mg in 1 ml) via the ear vein catheter, then euthanized by bolus IV injection of 30-50 ml of 4 M potassium chloride solution.

If a pig failed to develop subjective neurological signs after 1 h of observation, it ran on the treadmill, where its gait was assessed. Pigs with no discernible gait abnormality were categorized as "no neurological DCS". These pigs took no further part in the protocol.

Grading Severity of Neurological and Skin DCS

In previous studies using this model, the above procedures allowed the severity of neurological DCS to be crudely graded (5). We were unable to use the same criteria in this study because most of the DCS was ataxia and there were very few deaths. However, it was noted that some cases of ataxia were transient, while other cases lasted the entire hour of postdive observation. We therefore graded severity as "severe" for permanent ataxia, and "mild" for transient ataxia. For the skin DCS, again we were unable to use the previous grading system because very few lesions were larger than 20% of skin surface area.

Statistical Methods

Statistical comparison was by chi-squared analysis of discrete variables in 2 ft x 2 ft contingency tables, taking $p = 0.05$ as the threshold of significance. There is no accepted way of combining statistical analysis of both incidence and severity of DCS. Each of these results was therefore considered separately.

RESULTS

First Phase

Weight of Animals

The mean weight (±SD) for the control group was 17.2 (±1.5) kg. The mean weight for the deep stops group was 17.3 (±1.4) kg.

Neurological DCS

Of the pigs dived on the control profile, 13/31 (42%) were diagnosed as suffering from neurological DCS compared to 13/31 (42%) of the pigs decompressed on the deep stops profile.

Of the 13 affected pigs in the control group, 4 had transient ataxia, 8 had persistent ataxia, and 3 had weakness (2 of which also had persistent ataxia). Of the 13 affected pigs in the experimental group, 2 had transient ataxia, 10 had persistent ataxia, and 4 had weakness (of which 1 also had persistent ataxia). None died in either group.

There was thus no difference in the incidence of neurological DCS. Comparing severity (10/31 vs 8/31), chi-squared reveals no significant difference (Yates' corrected $c^2 = 0.078$; $p > 0.10$), though a slight trend appears in favor of the control profile.

Skin DCS

Of the 31 pigs in the control group, 6 (19%) developed skin DCS, none of them covering more than 20% of skin surface area compared to 7 (22%) in the deep stops group. Again, chi-squared analysis yields no significant difference (Yates' corrected $c^2 = 0.00$; $p > 0.10$).

Second Phase

Weight

The mean weight (±SD) for the control group was 20.0 (±1.6) kg. The mean weight for the deep stops group was 19.6 (±1.6) kg.

Neurological DCS

In the control group, 16/29 (55%) developed neurological DCS compared to 13/29 in the deep stops profile. Of the 16 affected pigs in the control group, 2 developed transient ataxia, 13 persistent ataxia, and 2 developed weakness (one pig having both persistent ataxia and weakness). Of the 13 affected pigs in the deep stops group, 2 suffered transient ataxia, 11 persistent ataxia, and 2 weakness (both pigs with weakness also having persistent ataxia). Comparison of these incidences by Yates' corrected chi-squared yields no significant difference.

Comparison of severe cases (13/29 vs 11/29) also yields no significant difference by chi-squared.

Skin DCS

In the control group, 11/29 (38%) developed cutaneous DCS. In the deep stops group, 13/29 (45%) developed cutaneous DCS. There is no significant difference in these proportions by Yates' corrected chi-squared. None of the skin lesions was over 20% of the pig's surface area.

DISCUSSION

Studies of native divers in the Torres Straits have revealed a surprisingly low number of bends cases on profiles that utilize deep stops but have a shorter total decompression time (3). U.S. Navy tables are viewed by these divers as time-consuming and unnecessary. In their presentation of the data, LeMessurier and Hills explain the possible benefits of using deep stops based on a thermodynamic model of decompression (3). Subsequently, others argued that Haldanian models do not really prevent bubble formation, but actually use long, shallow stops to "treat" bubbles produced by earlier, inadequate decompression stops (4). Theoretically, a deep stop could allow rapid off-gassing without a bubble ever forming, thus obviating the need for long, shallow stops.

These principles were briefly tested using a limited number of pig dives to develop a modified human table by computer manipulation (4). Although useful modified tables were developed by the procedure, the benefit of deep stops was not demonstrated in a rigorous statistical manner in this study.

The present study sought to follow up on this early work by a more thorough comparison of a conventional, Haldanian profile with a deep stop profile. In the first phase, as in the previously mentioned studies, comparing the incidences and severities of neurological and cutaneous DCS and introducing a deep stop into the profile seems to allow the decompression to be accelerated without any change in outcome. It could not be determined from this phase alone, however, if the benefit was truly conferred by the deep stop or if the 8 min could have been cut without the use of a deep stop. It was necessary to further test the deep stop profile by comparing it to a modified control profile that had the same TDT. In the second phase, the control profile was modified by making the last stop 8 min shorter. Comparing the incidences and severities of these 2 profiles also yielded no significant difference in the incidence of DCS, although a slight trend in favor of deep stops was seen. Any benefit of the deep stop was therefore below the Type II error of our study design. It is likely that there is a small benefit to the deep stops profile for neurological DCS, but demonstration of this benefit would require another 60 animals.

Interestingly, the deep stops profile also showed a slight trend of increased incidence for cutaneous DCS. This trend also did not reach statistical significance, but the fact that it was seen in both phases of the study causes one to consider whether the trend was really due to chance. This trend is in contrast to our previous studies quantifying both cutaneous and neurological DCS in pigs because the cutaneous variety of DCS usually shows a greater decrease in incidence relative to neurological DCS for a given intervention (5).

Although the results fail to show a large benefit to deep stops on this profile, the possibility of a significant benefit to using deep stops cannot be ruled out. Further work will be

necessary to demonstrate situations where deep stops would be useful and to quantitate any benefits.

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